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Case Report

First report of New Delhi metallo-β-lactamase 5 (NDM-5)-producing *Escherichia coli* from blood cultures of three leukemia patients

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SUMMARY

We report the first occurrence of New Delhi metallo-β-lactamase 5 (NDM-5) in carbapenem-resistant *Escherichia coli* isolated from blood cultures of three leukemia patients in northern China. These patients had at some time been hospitalized in the hematology department of the same hospital. All isolates were ST167 with identical pulsed-field gel electrophoresis patterns, suggesting a likely hospital transmission.

1. Introduction

New Delhi metallo-β-lactamase 1 (NDM-1) has received worldwide attention since it was first reported in *Klebsiella pneumoniae* recovered from a Swedish patient previously hospitalized in India. This is because of its severe resistance to almost all types of antibiotic, although NDM-1 has also captured worldwide attention because of its frequent association with additional resistance genes, presence in many common pathogens, and nosocomial spread.[1] As well as NDM-1, the first NDM-5-producing isolate was recovered from a patient with a recent history of hospitalization in India. As reported previously, NDM-5 differs from existing enzymes due to substitutions at positions 88 (Val→Leu) and 154 (Met→Leu), and reduces the susceptibility of *Escherichia coli* TOP10 transformants to extended-spectrum cephalosporins and carbapenems when expressed under its native promoter.[2] The detection of NDM-5 in three clinical isolates of carbapenem-resistant *E. coli* in northern China is reported herein.

2. Case report

The three NDM-positive *E. coli* isolates designated E1–E3 were recovered from blood cultures of three different hospitalized inpatients admitted to the Hematology Department of the General Hospital of Shenyang Military Command, Shenyang, China in 2014. The first isolate was recovered from a 56-year-old man who was diagnosed with acute leukemia and hospitalized from March 20 to April 24. The second was obtained from a 22-year-old male patient with B-lymphocytic leukemia who was hospitalized from April 2 to May 28. The third isolate was collected from a 63-year-old man with acute non-lymphocytic leukemia who was hospitalized on June 9 and died after 89 days of hospitalization. All three patients had undergone chemotherapy, blood cell transfusions, and prophylactic antibiotic therapy (imipenem and vancomycin).

The identification of the three isolates was conducted using the Vitek-2 AMS system (bioMérieux, France). The minimum inhibitory concentrations (MICs) of selected antimicrobials were determined by broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. All strains were resistant to the antibiotics, except one isolate that was susceptible to gentamicin. The resistance patterns are illustrated in Table 1.

All three isolates were positive for the modified Hodge test and EDTA–imipenem synergy test (Oxoid, UK), performed as recommended by the CLSI. The presence of bla_{NDM} (5′-GTCTGGCAGCA-CACTTCCTA-3′ and 5′-TAGTGCTCAGTGTCGGCATC-3′), bla_{KPC} (5′-TGTCACTGTATCGCCGTC-3′ and 5′-CTCAGTGCTCTACAGAAAACC-3′), and membrane protein genes (*omp*C and *omp*F)[3] was screened by PCR. The isolates were positive for bla_{NDM}, but negative for

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Table 1
Minimum inhibitory concentrations (μg/ml) for *Escherichia coli* isolates

	Antibiotics							
	CXM	CRO	CFP	CIP	GEN	IPM	MEM	TZP
CLSI resistance breakpoint	≥32	≥4	≥64	≥8	≥16	≥4	≥4	≥128/4
E1	≥512	≥512	≥512	≥512	128	16	64	≥512
E2	≥512	≥512	≥512	≥512	128	16	32	≥512
E3	≥512	≥512	≥512	≥512	≤1	16	32	≥512

CXM, cefuroxime; CRO, ceftriaxone; CFP, cefoperazone; CIP, ciprofloxacin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; TZP, piperacillin/tazobactam; CLSI, Clinical and Laboratory Standards Institute.

bla_{KPC} and without membrane absence; further sequencing identified them as bla_{NDM-5}-carrying.

Since the three patients had been hospitalized in the same department, and two of them had been hospitalized at the same time, multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were applied for analysis and molecular typing. The sequence type of all isolates was ST167, which is an internationally disseminated clone belonging to the ST10 complex. Their PFGE patterns were identical, with the same numbers of bands, and the corresponding bands were of the same apparent size.

3. Discussion

The three NDM-5-producing isolates were resistant to carbapenems and cephalosporins because the NDM enzyme hydrolyzes β-lactams. However, the resistance pattern to gentamicin was totally different. The major factor conferring aminoglycoside resistance is the aminoglycoside-modifying enzymes, and the intracellular location of these enzymes may have a role in determining the level of resistance.[4] Aminoglycoside-modifying enzyme genes may derive from genes encoding enzymes involved in normal cellular metabolism that always remain silent, and selective pressure of aminoglycoside usage may cause mutations and alter the expression of these enzymes. Hence, it is possible that the three isolates exhibited different resistance patterns to gentamicin.

These ST167 isolates with identical PFGE patterns were closely related to each other, but there was no significant evidence to verify whether the patients were cross-infected as they had been hospitalized in different wards and were treated by different doctors. Prophylactic antibiotic usage, chemotherapy, blood cell transfusions, and invasive procedures could all be involved in nosocomial infection. As reported, the NDM genes are located exclusively on plasmids and most plasmids readily undergo horizontal transfer, which implies an alarming potential for spread. Other studies have also found invasive procedures with bacterial-contaminated facilities to be associated with the transmission of NDM-producing *E. coli*. The selective pressure of the use of carbapenem antibiotics as initial prophylaxis has made carbapenem-resistant isolates dominant. All of the patients became infected with this NDM-5-producing *E. coli* during therapy. Finally, one patient died, one patient ceased treatment, and one was transferred to another hospital.

To date, NDM-5-producing *Enterobacteriaceae* have been reported in India (ST648), Algeria (ST2569), Japan (ST540), Spain (ST448), the UK (ST648),[2] USA (ST167), Australia (ST648), and West China (ST167)[5] since they were first reported. To our knowledge, this is the first report of NDM-5-producing *E. coli* isolated from cancer patients in China. Before this, a case of ST167 NDM-5-producing *E. coli* in a 75-year-old male patient with an acute exacerbation of chronic obstructive pulmonary disease was reported from a hospital in West China in 2014.[5] Although all four patients reported in China had no history of travel abroad, it remains unclear whether the ST167 NDM-5-positive isolates originated in China or not, and this requires further study. Some imported medical apparatus and instruments may have been the source of the infection. Cancer sufferers comprise a large proportion of hospitalized patients, and the use of chemotherapeutics and prophylactic antibiotics increases their chance of becoming infected.

This report will remind clinical doctors of the occurrence of 'superbugs'. We cannot avoid the use of antibiotics or the inevitable selective pressure resulting in the emergence of multidrug-resistant isolates, but we can control this through appropriate antibiotic usage and periodical monitoring. Furthermore, regular nosocomial infection control is absolutely necessary.

Conflict of interest: No conflict of interest to declare.

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